Epidemiology and Management of Xylella fastidiosa and other Exotic and Invasive Diseases and Insect Pests

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PROJECT OBJECTIVES:

1. Determine the epidemiology of exotic, emerging, re-emerging, and invasive diseases in California, including (but not necessarily limited to) Xf caused diseases of horticultural, agronomic, and ornamental crops.
2. Determine the nature and mechanism(s) of susceptibility/resistance to Xf infection and subsequent disease development in horticultural and agronomic crops, including (but not necessarily limited to) *Vitis* species and *Prunus* species.
3. Develop effective, economical, environmentally sound strategies to manage exotic, emerging, re-emerging, and invasive diseases, including (but not necessarily limited to) xylella diseases.

MAJOR ACCOMPLISHMENTS (2007–2010):

Advanced backcross generations of table grapes and raisins with high fruit quality and Pierce's disease resistance:

Introduction of Pierce's disease resistance from wild grape species into table grapes and raisins resulted in small berries with poor fruit quality. Repeated back-crossing generated table grapes and raisins that retain Pierce's disease resistance from *Vitis arizonica* but have fruit of high quality. Current advanced selections have the potential to be developed into new table grape and raisin cultivars with Pierce's disease resistance that will provide growers with an alternative to high quality cultivars that are susceptible to Pierce's disease.

Peach-almond hybrids are non-hosts of *X. fastidiosa* causing Almond Leaf Scorch disease:

Much of California's 700,000+ acres of almonds are susceptible to Almond Leaf Scorch, a disease caused by *X. fastidiosa*, the bacterium that also causes Pierce's disease of grapes. Studies were conducted to determine susceptibility of a peach-almond hybrid rootstock for almonds that boosts tree vigor. Research demonstrated that *X. fastidiosa* could not survive within the peach-almond hybrid. Use of peach-almond hybrid rootstocks in commercial orchards may reduce incidence of Almond Leaf Scorch disease by eliminating one possible source of the bacterium.

Identification of candidate genes involved in host plant resistance to Pierce's disease:

Cultivated table and wine grapes derived from the European grape (*Vitis vinifera*) are susceptible to infection by *Xylella fastidiosa*, the causal agent of Pierce's disease. Functional genomic and proteomic approaches identified genes involved in resistance to Pierce's disease. Among genes that were differentially expressed by susceptible and resistant progeny of crosses between *V. vinifera* and a wild grape, four candidate resistance genes were selected for further study. Information derived from these studies facilitated efficient selection of promising genetic backgrounds capable of limiting infection of *X. fastidiosa* in grapevines, a critical trait in the breeding of Pierce's disease resistant grapevines.

Arabidopsis thaliana as a model host for *Xylella fastidiosa*:

Novel control strategies for Pierce's disease of grapes may be derived from understanding complex genetic interplay between the pathogen (*X. fastidiosa*) and plant host. Research progress is hampered due to slow growth of grapes, long incubation periods between inoculation and expression of disease, and limited understanding of grape genes involved in susceptibility/resistance response to infection. *A. thaliana*, a fast-growing plant with a completely sequenced genome and a wide variety of defined ecotypes, was developed as a model host for *X. fastidiosa.* Two ecotypes of *A. thaliana* that differ in response to *X. fastidiosa* infection have been identified and are being screened for genes determining disease susceptibility/resistance. Knowledge of key host genes identified in the *A. thaliana-X. fastidiosa* model system may then be transferred to grapes and other plants of economic importance to define host-pathogen interactions and develop improved resistance to *X. fastidiosa* infection.

Development of a stable shuttle vector for delivery of genes to *Xylella fastidiosa*:
Novel targets for disease control strategies aimed at disrupting the ability of *X. fastidiosa* to induce Pierce's disease of grapes may be gleaned from molecular genetic studies in which specific *X. fastidiosa* genes are rendered non-functional by targeted mutation. To confirm that loss of ability to induce disease is due to mutation of the targeted gene, complementation of the mutant with a functional version of the gene is required. A stable plasmid shuttle vector for delivery of genes to *X. fastidiosa* was developed. Use of the shuttle vector greatly simplifies the process of complementing mutants expediting basic scientific research aimed at understanding the ability of *X. fastidiosa* to cause disease.

Ecological differences among glassy-winged sharpshooter populations indicates region-specific optimization of control practices:
Environmentally friendly alternatives to chemical control of the glassy-winged sharpshooter, a vector of *Xylella fastidiosa*, require an understanding of the insect's biology and ecology. Populations of glassy-winged sharpshooter in southern and central California are currently managed using essentially identical practices. Biological and behavioral characteristics of glassy-winged sharpshooter derived from separate populations in southern and central California were examined. Results indicate key differences among the two populations with respect to timing of reproduction and longevity. Based on these findings, management practices should be optimized specifically for each region such that control measures employed are more efficient.

Egg load and time since last egg laying affects behavior of the glassy-winged sharpshooter: Spread of insect-transmitted pathogens is largely dependent on population dynamics of the vector. To better understand oviposition (egg laying) patterns of the glassy-winged sharpshooter, a vector of *Xylella fastidiosa*, the effects of egg load (number of mature eggs carried by a female) on oviposition behavior were assessed. Results indicate that glassy-winged sharpshooter females were more likely to deposit eggs and accept a low-ranked oviposition host as egg load and time since last oviposition increased. New knowledge derived from the research will improve understanding of plant host utilization by glassy-winged sharpshooter and facilitate development of environmentally friendly control measures to reduce glassy-winged sharpshooter populations and, consequently, transmission of *X. fastidiosa*.

Elucidation of critical events in inoculation of *Xylella fastidiosa* to grapes by the glassy-winged sharpshooter:
X. fastidiosa, the bacterium causing Pierce's disease, is transmitted to grapes by the glassy-winged sharpshooter. The mechanism of insect inoculation of plants was examined. Results indicate that *X. fastidiosa* undergoes cyclic changes in population density in the glassy-winged sharpshooter and that inoculation of grapes involves flushing of bacteria from the insect precibarium. Subsequent deposition of bacteria into insect salivary sheaths associated with plant tissues appears to be the route by which *X. fastidiosa* gains access to grapevine xylem to establish infection. Knowledge gained from these studies will facilitate efforts to develop novel control strategies for Pierce's disease aimed at disrupting inoculation.

Molecular markers aid introgression of Pierce's disease resistance into table, raisin, and wine grape germplasm:
A quicker method to determine Pierce's disease resistance in plants is needed as evaluation under natural field (5 years) or controlled greenhouse (6 months) conditions are time consuming and costly. Introgression of resistance from a homozygous resistant form of *Vitis arizonica* (b43-17) was observed in a total of 4,321 seedlings from 83 different crosses of resistant *V. arizonica* selections and high-quality *V. vinifera* cultivars from the F1 and first and second modified backcross generations. Based on the presence of resistant molecular markers (VVIP26, ctg1026876 and VMC2a5), 1,683 PD resistant seedlings from wine, table, and raisin grape backgrounds were selected without prolonged field or greenhouse studies.

A new almond hybrid cures itself of almond leaf scorch bacteria during winter:
Reduced almond yield and tree mortality make almond leaf scorch disease a critical problem throughout California's 700,000+ acres. Through conventional plant breeding with a wild almond relative, a hybrid almond was developed that is better at overcoming almond leaf scorch disease through winter-curing as compared with the almond cultivar Butte. The new hybrid has edible almonds that are similar to Butte in color and kernel shape. This hybrid is significant because it demonstrates that a resistant almond variety can be developed by traditional breeding. The development of almond varieties resistant to this disease will be an economic benefit for the California almond industry.

The effect of grapevine xylem sap on *Xylella fastidiosa* culture:
Virtually all *Vitus vinifera* grape cultivars are susceptible to Pierce's disease and are not curable once infected with *X. fastidiosa*. However, some related grape species from the southern United States are resistant. To assess differences between resistant and susceptible grape species, the effect of xylem sap collected from resistant and susceptible grapevines on bacterial growth, biofilm formation, and cell aggregation was investigated. Media containing xylem sap from susceptible plants provided better support for bacterial growth than media supplemented with xylem sap from resistant plants. This study provides new insights of molecular basis of pathogenicity of xylem-limited bacteria and host resistance which facilitates target selection of promising genetic traits in the breeding of resistant grapevines.

Theoretical assessment of methods to reduce spread of *Xylella fastidiosa*:
Patterns of *X. fastidiosa* spread differ with insect vector species in California. A simulation model was used to assess the effectiveness of vine removal and insecticide treatments on slowing pathogen spread under different assumptions concerning the extent of within-field pathogen spread and vector reproduction in the affected crop. The results provide recommendations for improved control tactics.

Role of alfalfa in the epidemiology of diseases caused by *Xylella fastidiosa*:
Alfalfa has long been considered an important crop in the epidemiology of diseases caused by *X. fastidiosa* but limited research has been completed to assess importance of alfalfa as a source of *X. fastidiosa* and/or vectors. Weedy alfalfa fields were found to harbor large numbers of green sharpshooter, a known vector of *X. fastidiosa.*

Incidence of *X. fastidiosa* in alfalfa was low, due to vector preference for weeds over alfalfa. The results provide growers with an assessment on the potential for alfalfa to serve as a source of vectors and *X. fastidiosa* inocula.

Discovery and characterization of bacterial phage infecting *Xylella fastidiosa*:
Whether viruses (phage) may be used to control *X. fastidiosa* populations in infected plants has not been addressed. A novel phage of *X. fastidiosa* was identified by genomic analysis and electron microscopy. A survey of phage present in almond-infecting strains of *X. fastidiosa* was conducted. Discovery of *X. fastidiosa* phage provides a potential new alternative for biological control.

Retrospective assessment of glassy-winged sharpshooter invasion of California using surrogate virus population genetics:
A new virus species infecting the glassy-winged sharpshooter was isolated from California populations and characterized as a phytoreovirus species most closely related to rice dwarf virus. The complete genome of the new virus was determined and deposited in GenBank, the NCBI public database. Sequence polymorphism of the phytoreovirus was used as a surrogate marker to reconstruct population genetics of glassy-winged sharpshooter. Results indicated that the population in California experienced a recent genetic bottleneck consistent with limited introduction and was of an age consistent with introduction circa 1988 (2 years before the earliest detection). This new method also has utility for describing population characteristics of other invasive species.

Identification of California grape growing regions at risk for Pierce's disease epidemics:
Introduction of the glassy-winged sharpshooter vector of *Xylella fastidiosa* resulted in destructive epidemics of Pierce's disease in California. To describe why and explain where previous epidemics occurred and to predict where future epidemics are likely to occur, Geographic Information Systems maps on the distribution of grape (Pierce's disease susceptible) and citrus (glassy-winged sharpshooter reservoir host) in California and historical insecticide application databases were analyzed. These analyses identified which counties in California have the greatest overlap of citrus and grape and which counties applied the least amount of insecticides to citrus. Based on these results, Riverside, Kern, and Tulare counties (where previous outbreaks occurred) have the highest level of grape-citrus interface and appear to be at greatest risk for future epidemics. These findings will facilitate efforts to control Pierce's disease by identification of areas where vector populations would reach high levels in close proximity to vineyards.

A DNA-based assay to discriminate among G-genotype strains of *Xylella fastidiosa*:
G-genotype strains of Xyle*lla fastidiosa* are able to cause both Pierce's disease of grapevines and Almond Leaf Scorch disease. However, the ability to distinguish separate lineages of G-genotype strains is limited. Hyper-variation, due to differences in simple sequence repeats, within the *psbB* gene encoding a protease was identified and used to distinguish among G-genotype isolates. Pending verification of stability in simple sequence repeat differences, the assay may be useful for tracking individual *X. fastidiosa* lineages during epidemics.

Characterization of a novel plasmid in *Xylella fastidiosa*:
Xylella fastidiosa (causal agent of Pierce's disease and other vascular occlusion diseases of perennial plants) exists in nature as distinct strains able to exchange genetic material resident on extrachromosomal plasmids. Mulberry-infecting strains of *X. fastidiosa* were found to harbor a 25 kilobase pair (kbp) plasmid encoding Type IV secretion system genes known to facilitate DNA transfer among bacteria. Sequence analysis indicated that the 25 kbp plasmid shared significant sequence identity to the corresponding Type IV secretion system encoded by a 31 kbp plasmid resident in an unrelated bacterial species (*Verminephrobacter eiseniae*) that is a symbiont of earthworms. These results suggest that *X. fastidiosa* may acquire DNA from sources much more divergent than previously recognized. In addition, portions of the 25 kbp plasmid may be useful as components of a shuttle vector for delivery of foreign DNA to *X. fastidiosa*.

High-throughput detection of *Xylella fastidiosa* from plant tissues:
X. fastidiosa is responsible for Pierce's disease of grapevines and almond leaf scorch disease. Although the pathogen may be cultured from diseased plants, isolation of the pathogen from field samples is difficult and not suitable for evaluation of large sample sizes needed for epidemiological studies. A rapid and simple procedure for *X. fastidiosa* DNA extraction from diseased almond tissue was developed and found to be both reliable and suitable for high throughput PCR analysis. The technique will facilitate epidemiological studies in which *X. fastidiosa* infection status for a large number of samples are required to track pathogen spread and disease progression in the field.

Immunocytochemical protocols developed for detection of *Xylella fastidiosa* and glassy-winged sharpshooter watery saliva in grape tissue:
The mechanism by which the Pierce's disease bacterium, *Xylella fastidiosa*, is inoculated by sharpshooters is presently unknown. Evidence to date supports that bacteria are carried into the plant by injected watery saliva, which is invisible in classically stained plant tissues. Immunocytochemical protocols were developed for confocal microscopy to separately localize in grape tissue either *X. fastidiosa* cells or the primary enzymatic constituent of glassy-winged sharpshooter watery saliva, a beta 1,4-glucanase. These new methods will enable testing of the role of salivation in the inoculation mechanism, and may result in potential targets for disruption of pathogen transmission to grapes.

Complete genome sequence of two *Xylella fastidiosa* almond leaf scorch strains:
Genomic variation among strains of *X. fastidiosa* with distinct pathogenic properties was poorly described. With collaboration from Los Alamos National Laboratory, the genomes of two *X. fastidiosa* Almond Leaf Scorch strains were sequenced, annotated, and compared to four other strains for which complete genome sequences are available. Significant new information on genome rearrangement, gene complement, and prophage integration was found. The information will facilitate a better understanding of *X. fastidiosa* evolution and host range.

Management of almond leaf scorch disease:
Currently there are no effective management techniques that prevent almond trees from becoming infected *Xylella fastidiosa* and growers must decide to replace or keep Almond Leaf Scorch diseased trees. As the risk of tree-to-tree spread appears to be low, the decision to replace infected trees should focus on the loss of productivity due to infection. Yield and vitality of infected and uninfected almond trees were compared for two almond cultivars. Yields of Almond Leaf Scorch affected trees were significantly lower for both cultivars. To aid growers, a simple economic model was developed using field data to determine conditions under which replanting infected trees would increase returns.

Correlation of EPG X waveform with xylem penetration by sharpshooters:
The mechanism by which *Xylella fastidiosa* is inoculated to plants by sharpshooters is poorly understood. Sharpshooter feeding behavior during pathogen inoculation to plants was studied via electrical penetration graph (EPG) waveform analysis. The X waveform was identified as an indicator of sharpshooter mouth part (stylet) penetration into xylem, and is likely associated with inoculation of *X. fastidiosa* into the plant vascular system. Information on the inoculation mechanism will be used for predictive modeling and to develop varieties of grapes resistant to feeding by the glassy-winged sharpshooter.

A genomic approach to classify subgroups of *Xylella fastidiosa* strains:
There has been standardized procedure to classify *X. fastidiosa* strains below the species level. Over 100 16S rDNA sequences were analyzed. A combination of four single nucleotide polymorphisms (SNPs) sub-grouped all *X. fastidiosa* strains into four clusters, in agreement with the current knowledge of pathotypes and proposed sub-species. SNPs from eight other conserved genomic regions support this classification system. This research provides a simple, unambiguous method for identification of *X. fastidiosa* strains.

TECHNOLOGY TRANSFER/OUTREACH:

Technology Transfer:

- New scientific information transferred to end users through 61 peer-reviewed publications/proceedings and numerous presentations at meetings and symposia of scientific societies and stakeholder organizations.
- Electrical Penetration Graph Technology Workshop for insect feeding behavior (2007, 2009, 2010).
- Patent application filed: System and method for synchronizing waveform data with an associated video (with Bennett Electronics, 2009
- Patent application filed: Electrical penetration graph system. (with Bennett Electronics, 2009).
- vitisExpDB: a public database resource for grape functional genomics.
- nWayComp: a public tool for universal comparison of DNA and protein sequences.
- Deposited in Genebank:
    - Complete genome sequence of *X. fastidiosa* strains M12 and M23;
    - Numerous gene sequences of various *X. fastidiosa* strains;

- Complete genome sequence of four 25 kilobase-pair *X. fastidiosa* plasmids; and
- Complete genome sequences of nine GWSS virus strains.

EXTERNAL SUPPORT: (CURRENTLY ACTIVE AND DIRECTLY FOCUSED ON GRAPES)

- Breeding Pierce's Disease Resistant Table and Raisin Grapes and the Development of Markers for SEUS Sources of Resistance. University of California Pierce's Disease Research Grants Program. Reimbursable. [2008 – 2011].
- Breeding Pierce's Disease Resistant Table and Raisin Grapes and the Development of Markers from Seus Sources of Resistance. Consolidated Central Valley Table Grape Pest and Disease Control District. Trust. [2008 –2011].
- Design, Building, and Evaluation of a Production Version of Epg Monitor for Commercialization. Bennett Electronics. Cooperative Agreement

COLLABORATORS:

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RECENT PUBLICATIONS: (2007-2010)

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